

19



Europäisches Patentamt
European Patent Office
Office européen des brevets



11 Publication number: **0 603 726 A2**

12

EUROPEAN PATENT APPLICATION

21 Application number: 93120217.0

51 Int. Cl.⁵: **A23L 3/3517, A23L 1/303,
A61K 31/59**

22 Date of filing: 15.12.93

30 Priority: 21.12.92 US 993997

43 Date of publication of application:
29.06.94 Bulletin 94/26

84 Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC
NL PT SE

71 Applicant: Bristol-Myers Squibb Company
345 Park Avenue
New York, N.Y. 10154(US)

72 Inventor: Dube, David G.
7966 Owens Drive
Newburgh, Indiana 47630(US)
Inventor: Euber, John R.
14401 Honeysuckle Dr.
Evansville, Indiana 47711(US)
Inventor: Hansen, James W.
5600 Spring Park Dr.
Evansville, Indiana 47711(US)
Inventor: Mosler, Andrew C., Jr.
4510 Cromwell Dr.
Evansville, Indiana 47711(US)
Inventor: Napoll, Joseph L., Jr.
18 Hendricks Blvd.
Amherst, New York 14226(US)
Inventor: Richardson, Gwen G.
10266 Bourbon St.
Newburgh, Indiana 47630(US)

74 Representative: Kinzebach, Werner, Dr. et al
Patentanwälte
Reitstötter, Kinzebach und Partner
Postfach 86 06 49
D-81633 München (DE)

54 Nutritional compositions containing vitamin d esters.

57 The present invention is directed to nutritional compositions at an acidic to mid pH containing a vitamin D ester, an amino acid source, a carbohydrate source and a certain amount of a lipid source. The compositions have improved vitamin D stability upon storage.

EP 0 603 726 A2

FIELD OF THE INVENTION

The present invention concerns nutritional compositions containing esters of vitamin D.

5 BACKGROUND OF THE INVENTION

Vitamin D in the form of either ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃) has long been added to dairy products and infant formula to assure adequate nutritional supply. These foods vary in water content from a few percent as powders to about 90% or greater when normally consumed. Most of them
10 are of near neutral pH, although the pH of specialized formulas can vary. Milk-like infant formulas made with isolated soy protein or protein hydrolysates are also typically fortified with amounts of vitamin D similar to commercial whole milk. Some of these products, especially those made with hydrolyzed protein, are in an acidic pH range.

Although it is known that thermal or retort processing of nutritionally complete compositions often
15 results in a substantial, e.g. 20% loss in vitamin D activity (see M. Rechcigl, Jr., Ed., Handbook of Nutritive Value of Processed Food, Vol. I, CRC Series in Nutrition and Food, page 387 (1982)), storage stability after processing has been perceived in the industry to be quite satisfactory.

The official assay for vitamin D in food substances is the rat bioassay with its substantial intra and inter assay variation. Using this assay over the years, vitamin D in these substances has appeared to be stable
20 for considerable periods of time, even without refrigeration. For example, liquid infant formula products often have shelf lives of 12-18 months without any perceived significant loss of vitamin D activity.

We have discerned that vitamin D is much less stable upon storage in certain types of nutritional foods than previously thought. This stability problem has been solved through the use of certain esters of vitamin D in place of non-esterified forms of the vitamin.

It is taught in the art that vitamin D is slowly destroyed in an alkaline medium or in the presence of light and air, and that it is stable at a mid-pH (M. Rechcigl, Jr., Ed., Handbook of Nutritive Value of Processed Food, Vol. I, CRC Series in Nutrition and Food (1982)). Additionally, it has been taught that vitamin D is stable in corn oil, propylene glycol and milk, although vitamin D₂ was shown to deteriorate in propylene glycol when diluted in water (Tractor Jitco, Inc.: Scientific literature reviews on generally recognized as safe
30 (GRAS) food ingredients - vitamin D, PB-234 901, U.S. Dept. Commerce, July 1974; Huber, W. and Barlow, O.W.: Chemical and biological stability of crystalline vitamins D₂ and D₃ and their derivatives, J. Biol. Chem., 149:125-137 (1943)). It has also been disclosed that vitamin D is unstable in the presence of minerals (U.S. Patent 2,758,923) and in an aqueous environment (Fraser, D.R. and Kodicek, E.: Enzyme Studies on the Esterification of Vitamin D in Rat Tissues, Biochem. J. 109:457 (1968)).

Vitamin D esters are known to be synthesized *in vivo* in rats (see, for example, D. R. Fraser *et al.*, Biochem J., 106, pp 491-496 (1986)) and have been administered to rats, chickens, and Japanese quail (see W. A. Rambech, *et al.* Internat. J. Vit. Nutr. Res., 51, pp 353-358 (1981) and D. R. Fraser, *et al.*, Br. J. Nutr., 23, pp 135-140 (1969)). Heretofore addition of vitamin D esters to certain nutritional compositions to increase the stability of the vitamin has been unknown.
40

SUMMARY OF THE INVENTION

The present invention is directed to a nutritional composition, preferably nutritionally complete, at an acidic to mid-pH comprising (a) an ester of vitamin D, (b) an amino acid source, (c) a carbohydrate source,
45 and (d) greater than 0.5 grams of a lipid source per 100 kcal of total composition.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. The effect of pH on vitamin D₃ storage stability in a casein hydrolysate-based infant formula as
50 described in Example 1 in eight ounce cans.

Fig. 2. Comparison of vitamin D₃, vitamin D₃ acetate, vitamin D₃ octanoate, and vitamin D₃ palmitate stability in an infant formula stored at room temperature (22°C) and 37°C. "D" represents vitamin D₃; "D-palmitate" represents vitamin D₃ palmitate; "D-acetate" represents vitamin D₃ acetate; and "D-octanoate" represents vitamin D₃ octanoate.

Fig. 3. Vitamin D₃ and vitamin D₃ palmitate assay comparisons for HPLC and bioassay determinations. "D" represents vitamin D₃ and "D-palmitate" represents vitamin D₃ palmitate.

Fig. 4. Ratio of bioactivity divided by HPLC analysis for vitamin D₃ and vitamin D₃ palmitate.

Fig. 5. Comparison of stability for vitamin D₃ and vitamin D₃ palmitate in an infant formula stored at room temperature and at 37°C. (Experiment 322 as described in Example 8). "D-rt" represents formula containing vitamin D₃ stored at room temperature; "D-rt lab B" represents formula containing vitamin D₃ stored at room temperature and analyzed by Lab B; "D-37" represents formula containing vitamin D₃ stored at 37°C.; "DP-rt" represents formula containing vitamin D₃ palmitate stored at room temperature; "DP-rt-lab B" represents formula containing vitamin D₃ palmitate stored at room temperature and analyzed by lab B; and "DP-37" represents formula containing vitamin D₃ palmitate stored at 37°C.

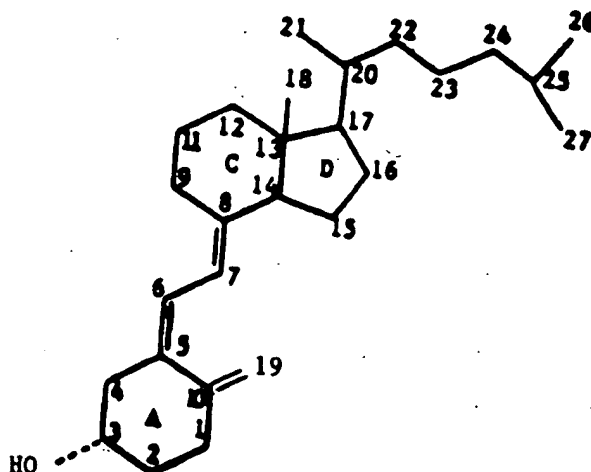
Fig. 6. Comparison of stability for vitamin D₃ and vitamin D₃ palmitate in an infant formula stored at room temperature and at 37°C. (Experiment 325 as described in Example 8). "D-rt" represents formula containing vitamin D₃ stored at room temperature; "D-rt lab B" represents formula containing vitamin D₃ stored at room temperature and analyzed by Lab B; "D-37" represents formula containing vitamin D₃ stored at 37°C.; "DP-rt" represents formula containing vitamin D₃ palmitate stored at room temperature; "DP-rt-lab B" represents formula containing vitamin D₃ palmitate stored at room temperature and analyzed by lab B; and "DP-37" represents formula containing vitamin D₃ palmitate stored at 37°C.

Fig. 7. Comparison of vitamin D₃ and vitamin D₃ levels in an infant formula at pre-retort, post-retort, and after 1, 2, 3, 4, and 5 months storage at room temperature and 37°C. "D 4oz rt" represents formula containing vitamin D₃ in 4 ounce glass containers stored at room temperature; "D 4oz 37" represents formula containing vitamin D₃ in 4 ounce glass containers stored at 37°C; "DP 4oz rt" represents formula containing vitamin D₃ palmitate in 4 ounce glass containers stored at room temperature; "DP quart rt" represents formula containing vitamin D₃ palmitate in one quart cans stored at room temperature; and "DP 4oz 37" represents formula containing vitamin D₃ palmitate in 4 ounce glass containers stored at 37°C.

Fig. 8. Vitamin D content of control (with vitamin D₃) and various starting levels of experimental (with vitamin D₃ palmitate) infant formulas as a function of storage time and temperature. The longer, upper curves represent storage at room temperature and the shorter, lower curves represent storage at 37°C. "DPalm-822 IU" represents formula containing vitamin D₃ palmitate at an initial concentration of 822 international units per quart; "DPalm-1120 IU" represents formula containing vitamin D₃ palmitate at an initial concentration of 1120 international units per quart; "DPalm-1372 IU" represents formula containing vitamin D₃ palmitate at an initial concentration of 1372 international units per quart; and "D-787 IU" represents formula containing vitamin D₃ at an initial concentration of 787 international units per quart.

DETAILED DESCRIPTION OF THE INVENTION

The vitamin D assay precision has been markedly improved using high performance liquid chromatography (HPLC) techniques. As we implemented these methods, it became apparent that vitamin D activity declined as a function of storage time in many nutritionally complete products. Different forms of vitamin D, e.g. vitamin D₂ and vitamin D₃, are similarly unstable. As used herein, the term "vitamin D" shall mean any of the antirachitic forms known in the art to be suitable for nutritional use such as vitamin D₁, vitamin D₂, vitamin D₃, vitamin D₄, vitamin D₅, vitamin D₆, and vitamin D₇. Preferred is vitamin D₃. The structure of vitamin D₃ is shown below with each carbon atom being numbered.



As used herein, the term "vitamin D activity" shall mean activity as determined in accordance with the HPLC procedure described in Example 6 hereof.

Various forms of non-esterified vitamin D are useful to prepare the esters of the invention such as those wherein the side chain (carbons 20-27) is modified. The vitamin D esters of the present invention are those wherein the hydroxyl group of carbon 3 is esterified with any esterifying acid suitable for use as food. As used herein, the term "ester" means a compound formed by the reaction of an acid and an alcohol with the elimination of water. The acid used to prepare the ester may be a C₁ to C₂₂, preferably C₂ to C₁₆, organic acid or an esterifying inorganic acid. Such organic acids are saturated or unsaturated aliphatic acids or aromatic acids and optionally contain additional functional groups such as amino, hydroxy, carboxy, and the like. Thus, the organic acids can be amino acids such as glycine or alanine; fatty acids such as acetic, octanoic or palmitic acids; hydroxylated acids such as lactic acid; acidic microbial inhibitor acids such as sorbic or benzoic acids or dicarboxylic acids such as succinic or fumaric acids. The acids may also be non-carboxylic acids such as sulfonic acids, phosphonic acids, or the like. Esterifying inorganic acids may also be used to form such esters as vitamin D sulfate, vitamin D phosphate, or the like. However, organic acids are preferred and fatty acids are most preferred. Fatty acid moieties of the invention can contain one to 22 carbon atoms, preferably 2 to 16 carbon atoms and are saturated or unsaturated. Specific examples of vitamin D esters useful herein include vitamin D acetate, vitamin D propionate, vitamin D caproate, vitamin D caprate, vitamin D laurate, vitamin D myristate, vitamin D palmitate, vitamin D stearate, vitamin D oleate, vitamin D linolenate, vitamin D arachidonate, vitamin D linoleate, vitamin D eicosapentaenoate, vitamin D docosahexaenoate, vitamin D benzoate, vitamin D lactate, vitamin D sorbate, vitamin D glycinate (alpha-amino acetate), vitamin D alanate (alpha-amino propionate), vitamin D succinate, vitamin D fumarate, vitamin D polyethylene glycol succinate, or a mixture thereof. Especially preferred are esters of vitamin D₃. Vitamin D₃ palmitate is most preferred.

The amount of the ester of vitamin D present in the composition of the invention per 100 kcal of total composition is typically about 0.1 µg to about 20 µg, preferably about 0.2 µg to about 20 µg, and more preferably about 1 µg to about 10 µg. In terms of international units (IU), the amount of the ester of vitamin D present in the composition of the invention provides per 100 kcal of total composition typically about 5 IU to about 600 IU, preferably about 10 IU to about 600 IU, and more preferably about 50 IU to about 300 IU.

Vitamin D esters of the invention can be prepared by methods known in the art or taught herein, for example, the acid halide, preferably the acid chloride (e.g. sulfonyl chloride, phosphoryl chloride, fatty acid chloride or the like) is reacted with the desired form of non-esterified vitamin D under Schotten-Baumann conditions using a strong base such as sodium hydroxide or under Einhorn conditions using a milder base such as pyridine. Other suitable esterification techniques can also be used such as using an appropriate dehydrating agent such as carbodiimide and the like. An example of reacting the desired form of non-esterified vitamin D with the desired fatty acid chloride is disclosed in Khim-Farm. Zh. 25(9) pp. 65-67 (1991).

The amount of water in the nutritional composition of the invention can vary widely since products can range from powders to ready-to-use liquid formulas; however, we have found that non-esterified vitamin D is generally less stable as a function of decreasing solids content. Thus, the water content can vary, for example, from about 2% to about 98%, based on the total weight of the composition. For powders a typical water content is about 2% to about 8%, preferably about 2% to about 5%; for liquid concentrate, a typical water content is about 65% to about 90%, preferably about 70% to about 85%; and for ready-to-use liquid, a typical water content is about 75% to about 98%, preferably about 85% to about 96%, said percentages being based on the total weight of the composition.

It has been found that the stability of vitamin D is related to the pH of certain nutritional compositions. Generally, the more acidic the pH composition, the less stable the non-esterified vitamin D which is surprising in view of the prior art which teaches instability at alkaline pH. As contemplated herein, the pH of the composition can be determined directly if in ready-to-use liquid form or, if in concentrate or powder form, the composition can be diluted with water to achieve a 68 kcal/dl caloric density and the pH then determined on the diluted product. The pH of the composition of the invention can vary from acid to mid pH; however, to achieve a more optimal protective effect of the vitamin D esters, pH of 7 or lower is preferred. The term "mid pH" refers to a pH range of 6.5 to 7.5. Typical pH ranges of the composition of the invention are about 4.0 to about 7.0, about 4.3 to about 6.8, about 5 to about 6.8 and about 5 to about 6. Typical pH ranges for specific products are, for example, about 5.5 to about 6.5, about 5.3 to about 5.7, about 6.2 to about 7.0, about 4.3 to about 4.7, and about 4.5 to about 6.5. A typical pH range for several applications is greater than 4.5 and up to 6.5.

Substituting the ester form of vitamin D for the nonesterified form results in marked improvement in storage stability. Non-esterified vitamin D in some nutritionally complete products has been determined by

the inventors to decline 30-50% in activity when stored at room temperature for 6 months. Declines are even more significant at increased storage temperatures. Thus, the present invention is also directed to a method for increasing the storage stability of a nutritional composition comprising supplementing said composition with an ester of vitamin D. Generally, the preferred embodiments applicable to the composition of the invention are also applicable to the method of the invention. It is preferred that the decline in vitamin D activity is improved at least 20% (more preferred is at least 30%, most preferred at least 50%) for the composition of the invention relative to a control composition having the same ingredients except for the corresponding non-esterified vitamin D when stored in a sealed container excluding light (e.g., in sealed metal cans) at 22 °C for six months. As an example of a 20% improvement contemplated in the previous sentence, if a control has a 50% decrease in vitamin D activity, a composition of the invention will have only a 40% or less decrease in vitamin D activity.

The nutritional composition of the present invention is preferably enteral; that is, it is designed for oral, intragastric, or transpyloric use. It is also preferred that the composition is nutritionally complete. By the term "nutritionally complete" is meant that the composition contains adequate nutrients to sustain healthy human life for extended periods. The composition of the invention may be an infant formula or adult nutritional composition. The composition also can be milk-based, soy-based, or based on other nutrients.

The composition of the invention contains ingredients which are designed to meet the nutritional needs of mammals, especially humans, such as a protein (amino acid) source, a lipid source, and a carbohydrate source. The composition of the invention can be an infant or adult nutritional composition. Typically milk, skim milk, casein, hydrolyzed casein, hydrolyzed whey protein, whey, vegetable protein concentrate (e.g. soy protein isolate), hydrolyzed vegetable protein (e.g. soy), animal oils, vegetable oils, starch, sucrose, lactose and/or corn syrup solids will be added to the composition to supply part or all of the amino acids and/or protein, lipid, and carbohydrate as well as other nutrients such as vitamins and minerals.

The amount of amino acid source per 100 kcal of total composition is typically about 1 g to about 10 g, preferably about 2 g to about 7 g; the amount of lipid source per 100 kcal of total composition is typically greater than 0.5 g up to about 10 g, preferably about 1 g to about 10 g, more preferably about 3 g to about 8 g, and most preferably about 3 g to about 6 g; and the amount of carbohydrate source per 100 kcal of total composition is typically about 5 g to about 23 g, preferably about 10 g to about 20 g.

The carbohydrate source can be any suitable carbohydrate known in the art to be suitable for use in nutritional compositions. Typical carbohydrate sources include sucrose, fructose, xylitol, glucose, maltodextrin, lactose, corn syrup, corn syrup solids, rice syrup solids, rice starch, modified corn starch, modified tapioca starch, rice flour, soy flour, and the like.

It has been found that the composition of the invention requires greater than 0.5 g of lipid source per 100 kcal of total composition in order to enhance the stability of vitamin D by use of vitamin D esters.

The lipid source can be any lipid or fat known in the art to be suitable for use in nutritional compositions. Typical lipid sources include milk fat, safflower oil, canola oil, egg yolk lipid, olive oil, cotton seed oil, coconut oil, palm oil, palm kernel oil, soybean oil, sunflower oil, fish oil and fractions derived thereof such as palm olein, medium chain triglycerides (MCT), and esters of fatty acids wherein the fatty acids are, for example, arachidonic acid, linoleic acid, palmitic acid, stearic acid, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, oleic acid, lauric acid, capric acid, caprylic acid, caproic acid, and the like. High oleic forms of various oils are also contemplated to be useful herein such as high oleic sunflower oil and high oleic safflower oil.

Medium chain triglycerides contain higher concentrations of caprylic and capric acid than typically found in conventional oils, e.g., approximately three-fourths of the total fatty acid content is caprylic acid and one-fourth is capric acid.

Moreover, it is also possible to use transesterified or interesterified lipids as part of the lipid source for the composition of the invention. Such lipids can be glycerol esters wherein the 1, 2, or 3 position is substituted with specific fatty acids for the purposes of obtaining the desired nutritional, physical, or functional characteristics of the lipid.

The amino acid source can be any protein and/or amino acid mixture known in the art to be suitable for use in nutritional compositions. Typical amino acids sources are animal protein, vegetable protein such as soy protein, milk protein such as skim milk protein, whey protein and casein, and amino acids (or salts thereof) such as isoleucine, phenylalanine, leucine, lysine, methionine, threonine, tryptophan, valine, and the like. A preferred amino acid source is hydrolyzed protein (protein hydrolysate) optionally supplemented with amino acids.

The protein hydrolysate useful in the invention may be any suitable protein hydrolysate utilized in a nutritional formula such as soy protein hydrolysate, casein hydrolysate, whey protein hydrolysate, other animal and vegetable protein hydrolysates, and mixtures thereof. The protein hydrolysate of the composi-

tion of the invention is preferably a soy protein, whey protein, or a casein hydrolysate comprising short peptides and amino acids, optionally supplemented with additional amino acids. In a preferred embodiment, the protein hydrolysate useful in the invention contains a high percentage of free amino acids (e.g. greater than 40%) and low molecular weight peptide fragments.

5 The hydrolyzed protein of the composition of the invention is also preferably supplemented with various free amino acids to provide a nutritionally balanced amino content. Examples of such free amino acids include L-tryptophan, L-methionine, L-cystine, L-tyrosine, and L-arginine.

Nutritionally complete compositions contain all vitamins and minerals understood to be essential in the daily diet and these should be present in nutritionally significant amounts. Those skilled in the art appreciate that minimum requirements have been established for certain vitamins and minerals that are known to be necessary for normal physiological function. Practitioners also understand that appropriate additional amounts (overages) of vitamin and mineral ingredients need to be provided to compensate for some loss during processing and storage of such compositions.

To select a specific vitamin or mineral compound to be used in the composition requires consideration of that compound's chemical nature regarding compatibility with the processing and shelf storage.

Examples of minerals, vitamins and other nutrients optionally present in the composition of the invention include vitamin A, vitamin B₆, vitamin B₁₂, vitamin E, vitamin K, vitamin C, folic acid, thiamine, inositol, riboflavin, niacin, biotin, pantothenic acid, choline, calcium, phosphorous, iodine, iron, magnesium, copper, zinc, manganese, chloride, potassium, sodium, selenium, chromium, molybdenum, taurine, and L-carnitine.

20 Minerals are usually added in salt form. In addition to compatibility and stability considerations, the presence and amounts of specific minerals and other vitamins will vary somewhat depending on the intended consumer population.

The composition of the invention also typically contains emulsifiers and stabilizers such as lecithin, (e.g., egg or soy), carrageenan, xanthan gum, mono- and diglycerides, guar gum, carboxymethyl cellulose, stearyl lactylates, succinylated monoglycerides, diacetyl tartaric acid esters of monoglycerides, polyglycerol esters of fatty acids, or any mixture thereof.

The composition of the invention may optionally contain other substances which may have a beneficial effect such as lactoferrin, nucleotides, nucleosides, immunoglobulins, and the like.

The composition of the invention may also optionally contain natural or artificial flavorants or colorants such as vanilla, chocolate, coconut, banana, strawberry flavors.

The osmolality of the liquid composition of the invention (when ready to consume) is typically about 100 to about 1100 mOsm/kg H₂O, more typically about 250 to about 700 mOsm/kg H₂O.

The composition of the invention can be sterilized, if desired, by techniques known in the art, for example, heat treatment such as autoclaving or retorting, irradiation, and the like.

35 The composition of the invention can be packaged in any type of container known in the art to be useful for storing nutritional products such as glass, lined paperboard, plastic, coated metal cans and the like. We have observed that non-esterified vitamin D is less stable when stored in glass containers as compared to metal can containers.

The invention is further illustrated by the following non-limiting examples. Percentages are by weight unless indicated otherwise.

EXAMPLE 1

45 A typical infant formula of the invention contains 86% water, 7.3% corn syrup solids, 2.2% casein enzymatically hydrolyzed, 2% corn oil, 1.5% modified corn starch, 0.5% soy oil, and less than 1% of each of the following: vitamins (vitamin A palmitate, vitamin D₃ palmitate, dl-alpha-tocopheryl acetate, phytonadione, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, vitamin B₁₂, niacinamide, folic acid, calcium pantothenate, biotin, ascorbic acid, choline chloride, inositol), minerals (calcium carbonate, calcium phosphate, magnesium oxide, ferrous sulfate, zinc sulfate, manganese sulfate, cupric sulfate, sodium iodide, potassium chloride, potassium citrate, sodium selenite), carrageenan, L-cystine, L-tyrosine, L-tryptophan, taurine, L-carnitine.

The combination of the various ingredients, when used at normal dilution, supply the nutrient levels listed in the following table.

<u>Nutrients</u>	Per 100 Calories (kcal) (5 fl oz)	<u>Per Quart</u>
Protein, g	2.8	18
Fat, g	3.9	25
Carbohydrate, g	13.4	86
Water, g	134	860
Linoleic acid, mg	2000	12800
Vitamins:		
Vitamin A IU	310	2000
Vitamin D ₃ IU	63	400
Vitamin E, IU	3.1	20
Vitamin K, μ g	15.6	100
Thiamine (Vitamin B ₁), μ g	78	500
Riboflavin (Vitamin B ₂), μ g	94	600
Vitamin B ₆ , μ g	63	400
Vitamin B ₁₂ , μ g	0.31	2
Niacin, μ g	1250	8000
Folic acid (Folacin), μ g	15.6	100
Pantothenic acid, μ g	470	3000
Biotin, μ g	7.8	50
Vitamin C (Ascorbic acid), mg	8.1	52
Choline, mg	13.3	85
Inositol, mg	4.7	30
Minerals:		
Calcium, mg	94	600
Phosphorus, mg	63	400
Magnesium, mg	10.9	70
Iron, mg	1.88	12
Zinc, mg	0.78	5
Manganese, μ g	31	200
Copper, μ g	94	600
Iodine, μ g	7	45
Sodium, mg	47	300
Potassium, mg	109	700
Chloride, mg	86	550

The pH of the formula is 5.3-5.7 and the osmolality is about 300 mOsm/kg H₂O, when diluted to 68 kcal/dl.

EXAMPLE 2

Another typical infant formula of the invention contains (on a dry basis) 32% corn syrup solids, 16.6% enzymatically hydrolyzed casein, 15.3% medium chain triglycerides (fractionated coconut oil), 10.5% modified corn starch, 9.9% dextrose, 5.6% corn oil, 3.5% soy oil, 3.5% high oleic safflower oil, and less than 2% of each of the following vitamins (vitamin A palmitate, vitamin D₃ palmitate, dl-alpha-tocopheryl acetate, phytonadione, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, vitamin B₁₂, niacinamide, folic acid, calcium pantothenate, biotin, ascorbic acid, choline chloride, inositol), minerals (calcium citrate, calcium phosphate, magnesium oxide, ferrous sulfate, zinc sulfate, manganese sulfate, cupric sulfate, sodium iodide, potassium citrate, potassium chloride, sodium selenite), L-cystine, L-tyrosine, L-tryptophan, taurine, L-carnitine.

The combination of the various ingredients, when used at normal dilution, supply the nutrient levels listed in the following table.

20

25

30

35

40

45

50

55

<u>Nutrients</u>	Per 100 Calories (kcal) <u>(5 fl oz)</u>	<u>Per Quart</u>
Protein, g	2.8	18
Fat, g	5.6	36
Carbohydrate, g	10.3	66
Water, g	134	860
Linoleic acid, mg	940	6000
Vitamins:		
Vitamin A, IU	380	2400
Vitamin D ₃ , IU	75	480
Vitamin E, IU	3.8	24
Vitamin K, μ g	18.8	120
Thiamine (Vitamin B ₁), μ g	78	500
Riboflavin (Vitamin B ₂), μ g	94	600
Vitamin B ₆ , μ g	63	400
Vitamin B ₁₂ , μ g	0.31	2
Niacin, μ g	1250	8000
Folic acid (Folacin), μ g	15.6	100
Pantothenic acid, μ g	470	3000
Biotin, μ g	7.8	50
Vitamin C (Ascorbic acid), mg	11.7	75
Choline, mg	13.3	85
Inositol, mg	4.7	30
Minerals:		
Calcium, mg	94	600
Phosphorus, mg	63	400
Magnesium, mg	10.9	70
Iron, mg	1.88	12
Zinc, mg	0.94	6
Manganese, μ g	31	200
Copper, μ g	94	600
Iodine, μ g	7	45
Sodium, mg	39	250
Potassium, mg	109	700
Chloride, mg	86	550

The pH of the formula is about 5.5 to about 6.5, and the osmolality is about 330 mOsm/kg H₂O, when diluted to 68 kcal/dl.

EXAMPLE 3

The effect of pH on vitamin D₃ stability in the casein hydrolysate-based infant formula of Example 1 (manufactured at pH 5.5) was studied at 37°C. The pH was adjusted by varying the amounts of organic acid (citric), inorganic acid (hydrochloric), or potassium hydroxide. Figure 1 shows the marked enhancement of stability as the pH is increased to 6.0, 6.4 and 6.8. However, this particular product suffered deterioration of other product qualities at the higher pH.

EXAMPLE 4

Vitamin D Palmitate Preparation

A 10 ml methylene chloride solution containing 1 gm of vitamin D₃, 0.78 gm of palmitic acid and 0.03 gm of 4-dimethylaminopyridine was stirred as 0.6 gm of 1,3-dicyclohexylcarbodiimide in methylene chloride was added slowly. The reaction was allowed to proceed for two hours at ambient temperature. Hexane was added, the urea by-product was removed by filtration, and the organic phase was washed with 0.2 N sodium hydroxide, 5% acetic acid in water, and water. The solvents were removed by evaporation under reduced pressure. The product was purified by chromatography through a column of water-deactivated alumina eluted with 1% tetrahydrofuran in hexane. No free vitamin D was detectable in the product which was analyzed to contain 52% vitamin D by spectroscopic absorption at 265 nm. This was confirmed after saponification by HPLC analysis for vitamin D.

EXAMPLE 5

Other Esters of Vitamin D

Vitamin D₃ acetate and vitamin D₃ octanoate were both prepared using the same procedures as that for vitamin D₃ palmitate where palmitic acid was replaced by acetic acid and octanoic acid, respectively. The three forms of esterified vitamin D₃ and non-esterified vitamin D₃ were incorporated into Pilot Plant batches of the protein hydrolysate product substantially as described in Example 1 (except for the form of vitamin D), pH adjusted, homogenized, sterilized (autoclaved) and stored at room temperature (22°C) and at 37°C in glass bottles. Vitamin D HPLC determinations (as described in Example 6) were made prior to sterilization, after sterilization and after storage at 37°C for 10 weeks. Due to apparent variability from container to container, the contents of 5 separate containers were pooled prior to analysis of the 10-week samples. Table 1 and Fig. 2 show that all three esters were comparably more stable after ten weeks at both room temperature and 37°C than was vitamin D itself.

Table 1

Vitamin D ₃ content of experimental products made with non-esterified vitamin D ₃ (control) and esterified forms of vitamin D ₃ as a function of storage time and temperature.					
Sample Age	Storage Temperature	Vitamin D ₃ Content (IU/QT)			
		D ₃	D ₃ acetate	D ₃ octanoate	D ₃ palmitate
Pre-sterilization ^a	-	805	698	741	633
3 week ^b	RT	488	435	511	501
10 Week ^c	RT	415	477	536	429
10 week ^c	37 ° C	152	257	302	259

a) Results are the average of 5-8 analyses from one bottle.

b) Results are the average of 3 analyses from one bottle.

c) Results are the average of 6 analyses from the contents of 5 bottles commingled.

EXAMPLE 6

Vitamin D Analyses

Vitamin D was analyzed by a HPLC assay as described by Sliva, M.A.; Green, A.E.; Sanders, J. K.; Euber, J.R.; Saucerman, J. R., Reversed-phase liquid chromatographic determination of vitamin D in infant formulas and enteral nutritionals. J. Assoc. Off. Anal. Chem. 1992, 75:566, except that saponification conditions of 70 ° C for 40 minutes were employed.

Bioactivity (using the official AOAC rat bioassay) was compared to high performance liquid chromatography results; replicate determinations were made for the non-esterified vitamin D in corn oil and in two Pilot Plant batches of formula and for the vitamin D palmitate in corn oil and in two other Pilot Plant batches of formula. Table 2 and Fig. 3 show the vitamin D₃ and vitamin D₃ palmitate assay comparisons for HPLC and bioassay determinations. Replicate determinations are shown and the HPLC levels appear to be slightly higher, but not significantly higher, than the bioassay results for both non-esterified and esterified vitamin D.

Ratios of vitamin D bioactivity divided by HPLC determination were computed for each of the six samples. Table 2 shows these data, ratios, and mean ratio, standard deviation and, standard error of the samples tested. Figure 4 displays these ratios as the mean and standard deviation. No significant difference was seen for the bioactivity of non-esterified vitamin D₃ compared to vitamin D₃ palmitate.

Table 2

Comparison of Rat Bioassay and High Performance Liquid Chromatography (HPLC) Results for Vitamin D ₃ and Vitamin D ₃ Palmitate.			
Vitamin Source	Bioassay	HPLC	Bioassay/HPLC
D in corn oil (IU/g)	14	16.1	0.768
	14	19.3	
	13	18.9	
		17.1	
D in Product, Batch 1 (IU/qt)	290	330	1.062
	390	310	
D in Product, Batch 2 (IU/qt)	320	370	0.871
	290	330	
Mean			0.900
S.D.			0.123
S.E.			0.087
D-Palmitate in corn oil (IU/g)	15	13.8	0.886
	14	16.9	
	13	17.6	
		14.9	
D-Palmitate in Product, Batch 3 (IU/qt)	470	740	0.719
	500	610	
D-Palmitate in Product, Batch 4 (IU/qt)	380	570	0.761
	450	520	
Mean			0.789
S.D.			0.071
S.E.			0.050
The mean Bioassay/HPLC ratio for Vitamin D does not differ significantly from that for Vitamin D-Palmitate.			

EXAMPLE 7

Long-term Storage Stability

Two additional experiments (identified as experiments 322 and 325) were performed using Pilot Plant batches of a liquid product substantially as described in Example 1 packaged in 8 ounce cans. For each experiment, a Pilot Plant batch of product was prepared with non-esterified vitamin D₃ as a control for the batch with vitamin D₃ palmitate. The product was retort sterilized and then stored at two different temperatures, room temperature and 37°C. Vitamin D content was measured by laboratory A by the HPLC method of Example 6 (except with slightly less stringent saponification conditions) before and after retort

sterilization and at 2, 4, 6, 9 and 12 months storage. Vitamin D in the product stored at room temperature was also independently measured by laboratory B (by the HPLC method of Example 6) before sterilization and after 5, 6, 7, 9, 10, and 12 months storage. Measurements by laboratory A at 12 months for experiment 322 and 9 and 12 months for experiment 325 were determined using an inappropriate method which was inadequate to fully recover the vitamin D₃ from vitamin D₃ palmitate in the product. Measurements by laboratory B from 0 through 4 months used inappropriate saponification conditions which were inadequate to fully recover the vitamin D₃ from vitamin D₃ palmitate in the product (laboratory B 0 time analyses were re-conducted using appropriate saponification conditions on frozen aliquots of pre-retort samples).

Table 3 and Figs. 5 and 6 show the results of the experiments demonstrating improved long-term stability for vitamin D₃ palmitate. Retort losses ranged from 21% to 29% and were similar for both non-esterified vitamin D and vitamin D palmitate in both experiments. A small loss from the post retort value over 9 months was observed for vitamin D palmitate stored at room temperature. Storage of vitamin D₃ palmitate at 37°C resulted in a loss of about 30-40% from post retort values by 6 months in both experiments. In marked contrast, storage of non-esterified vitamin D at 37°C led to losses approaching 80% from the post retort level by 6 months.

20

25

30

35

40

45

50

55

Table 3. Vitamin D Levels (IU/quart) in Pilot Plant Products Made of Vitamin D₃ or with Vitamin D₃ Palmitate and Stored at Room Temperature or at 37°C and Tested by Two Laboratories.

Sample Age	Experiment 322										Experiment 325				
	Room Temperature					37°C					Room Temperature				
	D					D					D				
	Lab A	Lab B	Lab A	Lab B	D-Palmitate	Lab A	Lab B	D	D-P		Lab A	Lab B	D-Palmitate	D	D-P
Pre-retort	900	939	773	829	773	900	845	787	773		787	865	966	787	865
Post-retort	637		569		569	637		624	569		624	674		624	674
2 months	550		600		600	400		450	543		450	676		270	520
4 months	500		550		550	190		420	367		420	707		130	420
5 months		509		628						437			742		
6 months	433	437	543	653		162	414	327	373		327	567	738	143	317
7 months		347		593			324						635		
9 months	330	349	487	572			335	327					695		
10 months		329		560			247						650		
12 months	260	253		497			210	280					603		

Experimental production batches of product described in Example 1 were prepared containing either vitamin D₃ (control) or vitamin D₃ palmitate. The control product was packaged in 4 oz glass bottles and product with vitamin D₃ palmitate was stored in 4 oz glass bottles and in quart cans. The 4 oz bottled products were stored at room temperature and at 37°C and the quart cans at room temperature only. All samples were analyzed by laboratory A.

Table 4 and Fig. 7 show vitamin D levels post-retort, and after 1, 2, 3, 4 and 5 months storage at room temperature and 37°C. These results confirm the results of the pilot experiments, i.e., that vitamin D palmitate is more stable than vitamin D.

Table 4
Vitamin D (IU per 100 Kcal) in Experimental Production Batches of Product
20 Calories/fluid ounce (67.6 kilocalories/deciliter)
Stored at room temperature (RT) and at 37°C

Batch	Initial (Pre- Retort)	Initial (Post- Retort)	1 month		2 months		3 months		4 months		5 months	
			RT	37°C	RT	37°C	RT	37°C	RT	37°C	RT	37°C
Vitamin D 4 oz	118.2	99.2	89	54	73.6	33.9	67.8	24.3	57.7	14.5	56.6	18.
D-Palmitate 4 oz	125.2	122	104	74	109.5	59.4	97.7	43.8	101	46.8	101	35
D-Palmitate quart	125.2	121	109	*	115	*	112	*	109	*	109	*

*Not stored at this temperature.

EXAMPLE 8

Nutritionally complete infant formulas were prepared which have the composition substantially as described in Example 1 except for variation in the vitamin D source. Control (with non-esterified vitamin D₃) and experimental products (with vitamin D₃ palmitate) were prepared. Vitamin D₃ palmitate was added at three levels to product. The products were stored at room temperature and at 37°C. Vitamin D₃ analyses were performed pre-retort, post-retort, and at various times up to 38 weeks using the HPLC procedure described in Example 6. Mean values are presented in Table 5 and percent of pre-retort value in Fig. 8. The stability at all levels of vitamin D palmitate was comparable and significantly greater than for vitamin D (non-esterified) when stored at the same temperature.

Table 5

Vitamin D ₃ content of control (vitamin D ₃) and experimental (vitamin D ₃ palmitate) products as a function of storage time and temperature ^a .					
Sample Age	Storage Temperature	Vitamin D ₃ Content (IU/QT)			
		D ₃ Control	D ₃ Palmitate Expt. 1	D ₃ Palmitate Expt. 2	D ₃ Palmitate Expt. 3
Pre-sterilization	-	787	822	1120	1372
3 week	RT	475	636	837	1047
5 week	37 °C	354	473	660	785
9 week	RT	482	588	813	1058
9 week	37 °C	217	413	496	775
18 week	RT	363	582	697	945
18 week	37 °C	54	245	237	399
38 week	RT	245	452	704	812

a) Results the average of 3 analyses from one bottle (0-18 weeks) or 6 analyses representing 2 bottles and 3 samples/bottle (38 weeks).

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

Claims

1. A nutritional composition at an acidic to mid pH range comprising (a) an ester of vitamin D, (b) an amino acid source, (c) a carbohydrate source, and (d) greater than 0.5 grams of a lipid source per 100 kcal of total composition.
2. The composition of Claim 1 wherein said composition is nutritionally complete.
3. The composition of Claim 1 or 2 wherein said composition is for enteral feeding.
4. The composition of any one of the preceding claims having a pH of 7 or lower.
5. The composition of Claim 1 wherein said pH range is about 4 to about 7; preferably about 4.3 to about 6.8; more preferably about 5 to about 6.8; and in particular about 5 to about 6.
6. The composition of any one of the preceding claims further comprising water in an amount of about 2 % to about 98 %, preferably about 65 % to about 98 %, more preferably about 65 % to about 90 % and in particular about 75 % to about 98 % based on the total composition weight.
7. The composition of Claim 1 of any one of the preceding claims wherein said ester of vitamin D is a fatty acid ester of vitamin D or vitamin D₃.
8. The composition of any one of the preceding claims wherein said ester of vitamin D is vitamin D acetate, vitamin D propionate, vitamin D myristate, vitamin D palmitate, vitamin D stearate, vitamin D oleate, vitamin D linolenate, vitamin D arachidonate, vitamin D linoleate, vitamin D caprate, vitamin D caproate, vitamin D laurate, vitamin D eicosapentaenoate, vitamin D docosahexaenoate, vitamin D benzoate, vitamin D lactate, vitamin D sorbate, vitamin D glycinate, vitamin D alanate, vitamin D succinate, vitamin D fumarate, vitamin D polyethylene glycol succinate, vitamin D sulfate, vitamin D phosphate, or a mixture thereof, or vitamin D₃ acetate, vitamin D₃ propionate, vitamin D₃ myristate,

- vitamin D₃ palmitate, vitamin D₃ stearate, vitamin D₃ oleate, vitamin D₃ linolenate, vitamin D₃ arachidonate, vitamin D₃ linoleate, vitamin D₃ caprate, vitamin D₃ caproate, vitamin D₃ laurate, vitamin D₃ eicosapentaenoate, vitamin D₃ docosahexaenoate, vitamin D₃ benzoate, vitamin D₃ lactate, vitamin D₃ sorbate, vitamin D₃ glycinate, vitamin D₃ alanate, vitamin D₃ succinate, vitamin D₃ fumarate, vitamin D₃ polyethylene glycol succinate, vitamin D₃ sulfate, vitamin D₃ phosphate, or a mixture thereof.
9. The composition of Claim 8 wherein said fatty acid ester of vitamin D is vitamin D₃ palmitate.
 10. The composition of any one of the preceding claims wherein the amount of component (d) is about 1 g to about 8 g, per 100 kcal of total composition, preferably about 3 g to about 6 g per 100 kcal of total composition.
 11. The composition of any one of the preceding claims wherein the amount of component (a) is about 0.1 µg to about 20 µg, the amount of component (b) is about 1 g to about 10 g, the amount of component (c) is about 5 g to about 23 g, and the amount of component (d) is about 1 g to about 10 g per 100 kcal of total composition, or preferably the amount of component (a) is about 1 µg to about 10 µg, the amount of component (b) is about 2 g to about 7 g, the amount of component (c) is about 10 g to about 20 g, and the amount of component (d) is about 3 g to about 8 g per 100 kcal of total composition.
 12. The composition of any one of the preceding claims wherein said amino acid source comprises soy protein, whey protein, casein, skim milk protein or protein hydrolysate.
 13. The composition of any one of the preceding claims wherein said amino acid source is casein hydrolysate, optionally supplemented with one or more amino acids selected from the group consisting of L-tryptophan, L-methionine, L-cystine, L-tyrosine, L-arginine.
 14. The composition of any one of the preceding claims wherein said lipid source comprises medium chain triglycerides.
 15. The composition of any one of the preceding claims further comprising one or more substances selected from the group consisting of minerals, other vitamins, emulsifiers, stabilizers and a mixture thereof, said emulsifiers or stabilizers preferably being one or more selected from the group consisting of carrageenan, lecithin, monoglycerides, diglycerides, polyglycerol esters of fatty acids, xanthan gum, guar gum, carboxymethyl cellulose, stearyl lactylates, succinylated monoglycerides, and diacetyl tartaric acid esters of monoglycerides.
 16. The composition of any one of the preceding claims wherein said carbohydrate source is, sucrose, glucose, fructose, xylitol, maltodextrin, lactose, corn syrup, corn syrup solids, rice syrup solids, rice starch, modified corn starch, modified tapioca starch, or a mixture thereof and said lipid source is safflower oil, high oleic safflower oil, soy oil, egg yolk lipid, olive oil, cotton seed oil, coconut oil, palm kernel oil, canola oil, palm oil, sunflower oil, high oleic sunflower oil, fish oil, palm olein oil, medium chain triglycerides, esters of fatty acids wherein the fatty acids are selected from the group consisting of arachidonic acid, linoleic acid, palmitic acid, stearic acid, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, oleic acid, lauric acid, capric acid, caprylic acid, and caproic acid, or a mixture thereof.
 17. The composition of any one of the preceding claims in liquid form and for human consumption.
 18. A compound selected from the group consisting of vitamin D caprate, vitamin D propionate, vitamin D benzoate, vitamin D lactate, vitamin D sorbate, vitamin D glycinate, vitamin D alanate, vitamin D succinate, vitamin D fumarate, vitamin D polyethylene glycol succinate, vitamin D sulfate, and vitamin D phosphate, vitamin D₃ caprate, vitamin D₃ propionate, vitamin D₃ benzoate, vitamin D₃ lactate, vitamin D₃ sorbate, vitamin D₃ glycinate, vitamin D₃ alanate, vitamin D₃ succinate, vitamin D₃ fumarate, vitamin D₃ polyethylene glycol succinate, vitamin D₃ sulfate, and vitamin D₃ phosphate, or mixtures thereof, preferably vitamin D₃ octanoate.
 19. A method for increasing the storage stability of a nutritional composition comprising supplementing said composition with an ester of vitamin D.

20. The method of Claim 19 wherein said nutritional composition is as defined in Claims 1 to 6 or 10 to 17.

21. The method of Claim 20 at a pH of 7 or lower wherein said ester of vitamin D is a fatty acid ester of vitamin D, preferably vitamin D₃ palmitate and is present in an amount of about 0.1 µg to about 20 µg per 100 kcal of total composition, the amount of component (a) is about 1 g to about 10 g per 100 kcal of total composition, the amount of component (b) is about 5 g to about 23 g per 100 kcal of total composition, and the amount of component (c) is about 1 g to about 6 g per 100 kcal of total composition.

22. The method of Claim 21 having an improvement in storage stability of at least 20 % and in particular of at least 50 % relative to a control composition having the same ingredients except for the corresponding form of non-esterified vitamin D when stored in a sealed container excluding light at 22 °C for six months.

Vitamin D Stability at Different pH's Product (Example 1) stored at 37C

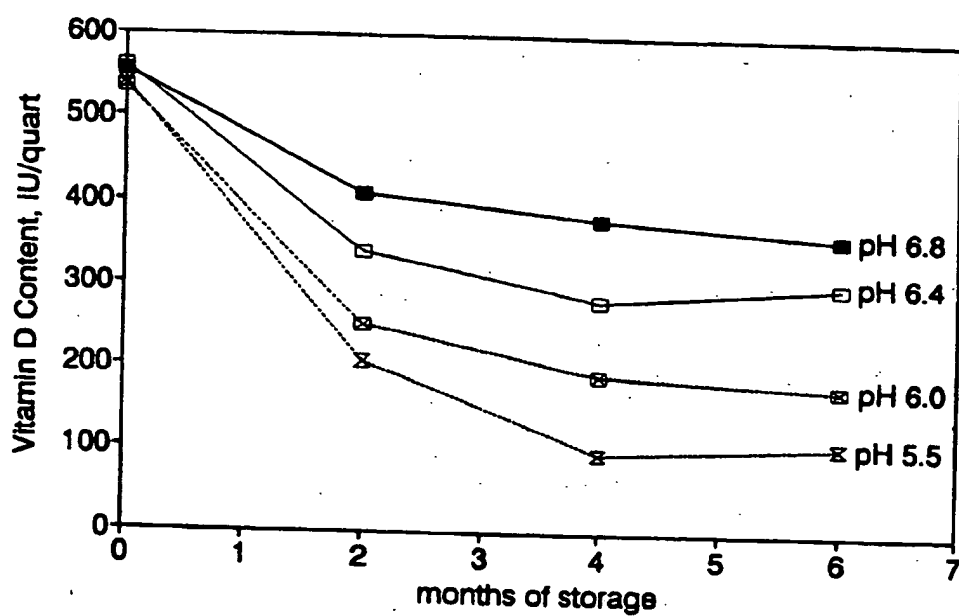


Figure 1

Stability of Different Vitamin D Esters Stored at room temperature (rt) & 37C

in Experimental Products (Example 1)

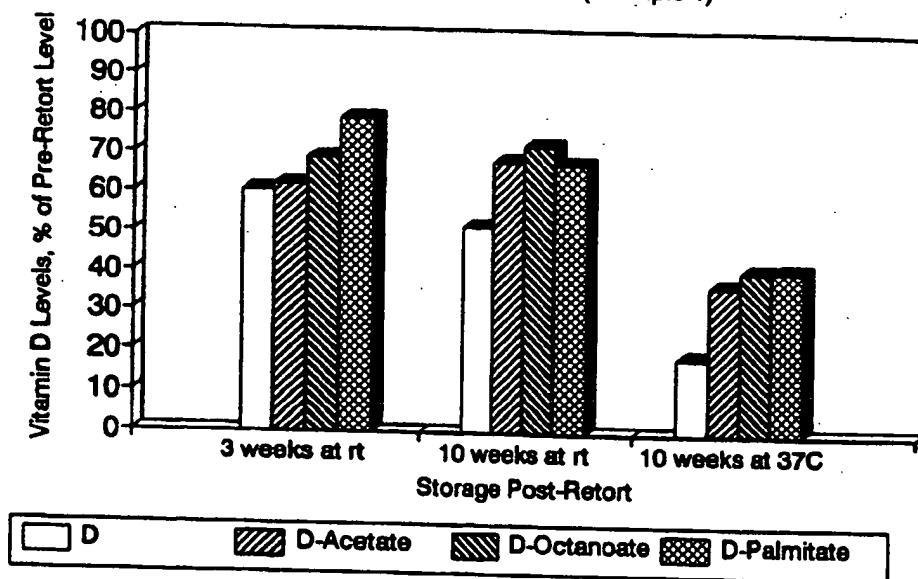


Figure 2

Vitamin D and Vitamin D Palmitate Assay Comparison

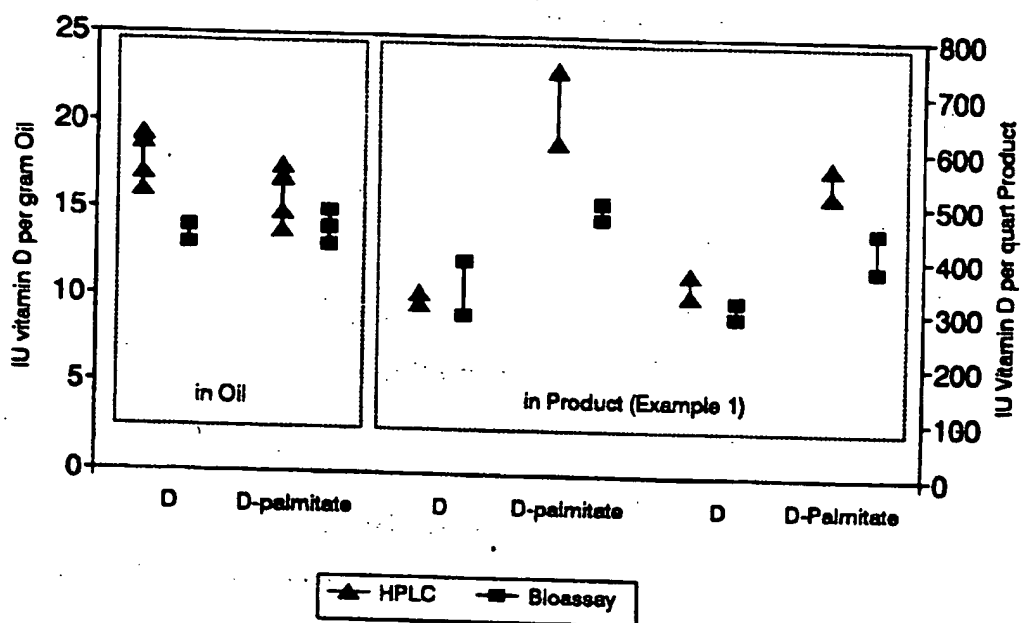
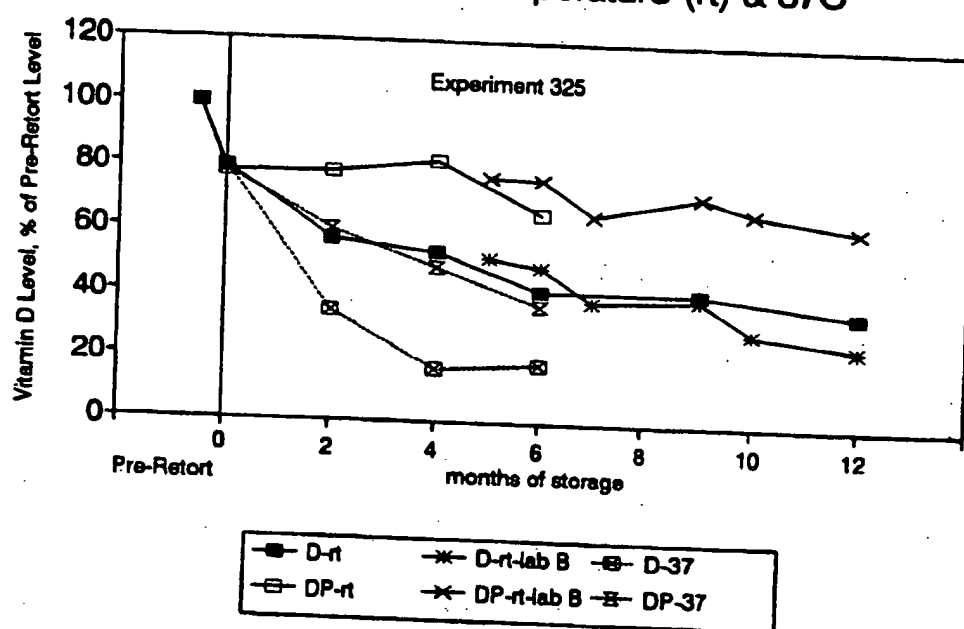


Figure 3

Vit. D & Vit. D Palmitate Stability storage at room temperature (rt) & 37C

**Figure 6**

Vit. D & Vit. D Palmitate Stability storage at room temperature (rt) & 37C

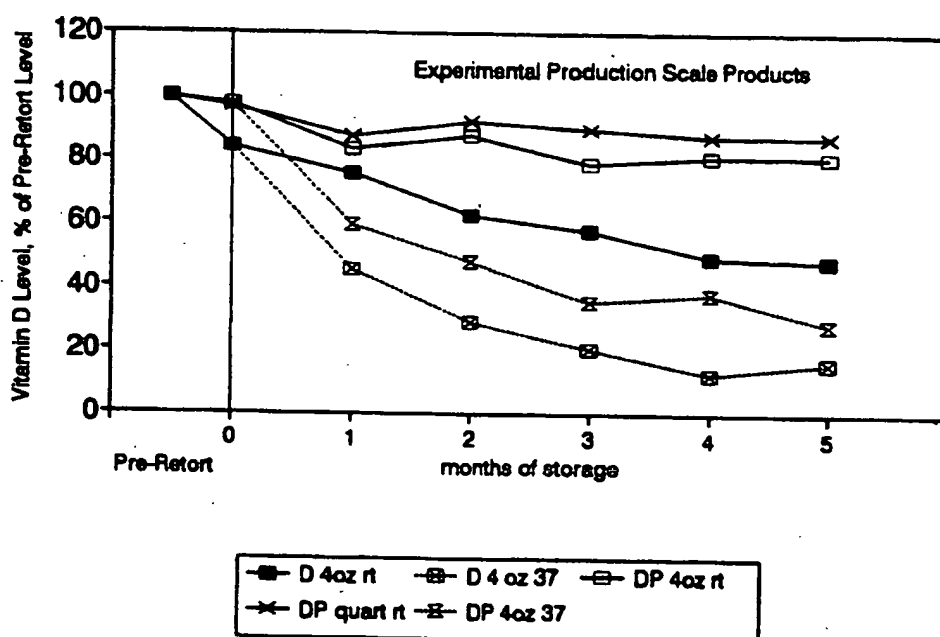


Figure 7

Vit. D & Vit. D Palmitate Stability

Different Levels of Vitamin D

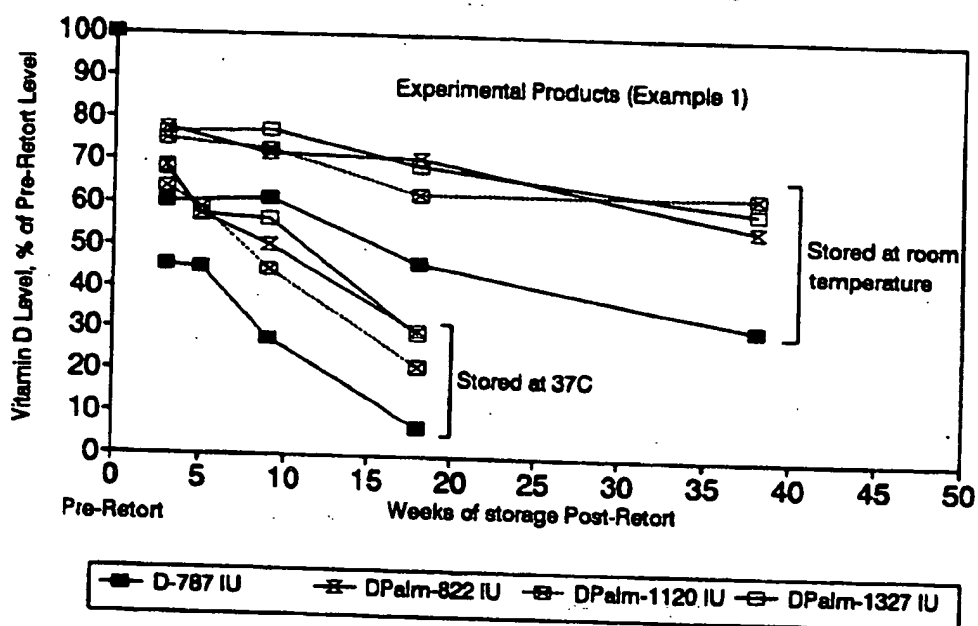


Figure 8